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Observations on Egg Hatching in the Estuarine Crab *Sesarma haematocheir*[1]

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ABSTRACT: A female of the terrestrial crab *Sesarma haematocheir* incubates 30,000–50,000 eggs on her abdomen. After 1 month of embryonic development, zoeae larvae are released into estuarine waters within 3–5 sec by means of vigorous fanning motions of the abdomen. Hatching (breakage of the outer egg membrane) occurs on land just before larval release. The release behavior itself does not cause rupture of the egg case, nor has the presence of a "hatching enzyme" been obviously demonstrated. Hatching seems to be induced by mechanical rupture of the egg case. The pressure responsible for hatching may be produced either by the larva itself, or by osmotic swelling of thin inner membranes encasing the larva, although neither of these hypotheses is sufficient at present to explain the complete hatching mechanism. If hatching is explained by such mechanisms, then there remains the question of how hatching is synchronized among the large number of embryos attached to the female. Hatching of detached embryos is synchronized to some extent, but the degree of synchronization is less than that occurring in the larvae carried by the female. This observation suggests that stimuli from the female are important in establishing highly synchronized hatching. The ecological significance of the hatching system is also discussed.

NEWLY OVIPOSITED EGGS of decapod crustaceans are attached to the abdominal appendages of the females, where they are ventilated during development by movements of the pleopods. When embryonic development is completed, hatching (egg-membrane rupture or breakage) occurs. Hatching in marine species occurs during the larval stage, and larval release is associated with vigorous abdominal fanning behavior (e.g., DeCoursey 1979).

A remarkable feature of larval release is synchrony with environmental cues such as day-night, tidal, or lunar cycles. Nocturnal release rhythms have been observed for a number of crustaceans. In the lobster, *Homarus*, larvae are released by the female a few minutes after darkness on successive days (Ennis 1973, Branford 1978). Hatch time of the fiddler crab, *Uca*, is closely correlated with high tide at night (DeCoursey 1979). Precisely timed rhythms in larval release implies that hatching time is also synchronized within a batch of eggs attached to a female.

Little is known about the hatching mechanism in Crustacea. It may be mechanical, nonmechanical, or a combination of both. Osmotic hatching has been suggested for a number of crustaceans. In this hypothesis, the rigid outer egg membrane may be burst by osmotic pressure inside the thin inner membrane (Yonge 1937, Marshall and Orr 1954, Davis 1965), or by pressure caused by the swelling larva itself (Davis 1964).

The crab *Sesarma haematocheir* burrows on land surrounding small estuaries in Japan and along the coastlines of Southeast Asia. In early summer, a female incubates 30,000–50,000 eggs in her folded abdomen for ca. 1 month. She emerges at riverside or seashore where the zoeae are released. About the time of high tide at night, the females immerse themselves in water and vigorously flex their abdomens as clouds of newly hatched larvae are swept away. This larval release behavior is completed in 3–5 sec, and all the zoeae are released from the pleopods. After larval release, the females return to land and incubate

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the next clutch, which will appear within a few days. Each female incubates and releases larvae two or three times between July and September.

It was obvious from field observations that embryos carried by the female had not yet hatched when the female appeared on the shore. That the behavior associated with release is an extremely short-lived event suggests several possible mechanisms enabling larval emergence from the egg membranes: (1) physical stimuli from the female during release behavior (i.e., vigorous fanning movements of pleopods and abdomen cause breakage of the egg membrane); (2) a hatching enzyme secreted by the zoea itself or the female that digests the egg case; or (3) some other mechanism that controls time of hatching. To determine which of the possibilities is correct, field studies were carried out to determine when and where the larvae hatch, and the hatching process was examined by microscope. The results raised questions about synchrony in hatching, and investigations were focused on whether detached eggs hatch with the same synchrony as observed when the eggs are attached to a female.

MATERIALS AND METHODS

Field Studies

This study was conducted at two sites on the coast of the Inland Sea, Japan: the margin of the outlet of a small river at Kasaoka where larval release activity had been observed (Saigusa 1982), and at a similar place at Ushimado, Okayama Prefecture, 70 km east of Kasaoka. A scanning electron microscope was utilized at the latter site.

Larval release activity of *S. haematocheir* coincides with high tide at night (Saigusa 1982, 1985). To find out when the embryos hatch, ovigerous females approaching riverside were captured about dusk. They were confined in empty plastic buckets and examined for hatching. Hatching was distinguished by the following features: (1) at hatching, the whole sponge or egg mass becomes somewhat sticky (that is, when touched with a finger, the hatched zoeae stick to it); and (2) females with sticky eggs froth slightly at the mouth. The sponge was frequently checked and the number of females with hatched larvae recorded at intervals of about 15 min. Collecting and counting crabs was accomplished with a hand-held flashlight.

Stereomicroscope Observations

Ovigerous females were captured from the field at Ushimado or Kasaoka and brought to the laboratory. Small samples of eggs (embryos folded within the membranes) were removed from females expected to release larvae that night. The samples were placed in a laboratory dish containing a small quantity of diluted seawater. Salinity was not recorded as there is no clear evidence for osmotic effects, even in distilled water. Observations on hatching were made with a stereomicroscope magnifying 20–90 times.

Scanning Electron Microscope Observations

The empty egg cases remaining immediately after larval release, and recently hatched larvae detached from the pleopods of females with forceps, were prepared for the scanning electron microscope (SEM) to analyze them for evidence of a hatching enzyme, which would be indicated by dissolution on the egg membrane enclosing the embryo. The samples were fixed for 2 hr in a solution containing 2.5% glutaraldehyde in 0.1 M Na cacodylate buffer (pH 7.4). After fixation they were washed twice in a 1:1 mixture of 0.1 M Na cacodylate buffer and distilled water, dehydrated through a graded series of ethanol, and dried in a Hitachi HCP-1 critical point apparatus. They were mounted on metallic stubs with double-stick tape, plated with gold, and examined.

Hatching of Detached Embryos

Detached embryos were examined to determine whether they hatch at the same time as those attached to the female. Crabs with embryos estimated to hatch within 2 days (based on the color of embryos) were chosen for these experiments. Detached embryos in

nonaerated water, detached embryos with aeration and water turbulence, and embryos attached to the female were utilized in the experiments. For the first condition, 300–1000 embryos were removed 3–3.5 hr before dark and placed in plastic cases (8 cm in diam., 6 cm in depth) approximately one-third filled with dilute seawater (10‰). At 30-min intervals, the swimming zoeae were counted and removed by pipette. A small flashlight (with two 1.5-V batteries) covered with red cellophane was used during the dark period.

All experiments on detached embryos were performed in experimental rooms in which light and temperature were controlled. A 15:9 (L:D) photoperiod similar to that in the field was employed. Temperature was maintained at 23 ± 1.5°C. As shown in Figure 1, some detached embryos were suspended on a horizontal nylon thread stretched across the center of an opening of a small plastic case, and the case was submerged; at 0.5-hr intervals the case was transferred to another beaker that contained a similar quantity of diluted seawater. The berried crabs were separately confined in vessels containing diluted seawater (ca. 1 cm deep), and hatching and larval release were monitored simultaneously.

RESULTS

Synchronous Hatching and Larval Release

The purpose of the field studies was to determine when and where the embryos hatched. When females were collected at riverside about dusk, hatching had not yet occurred. Hatching from free animals began just after sunset and reached a peak when the tide was highest (Figure 2, upper panel). Hatching was estimated to occur within a few minutes, and perhaps in less than 1 min for most of them, although it was impossible to determine the exact time required for completion of hatching. As soon as hatching was completed, the female produced a small amount of froth at the mouth. Subsequently, when the female

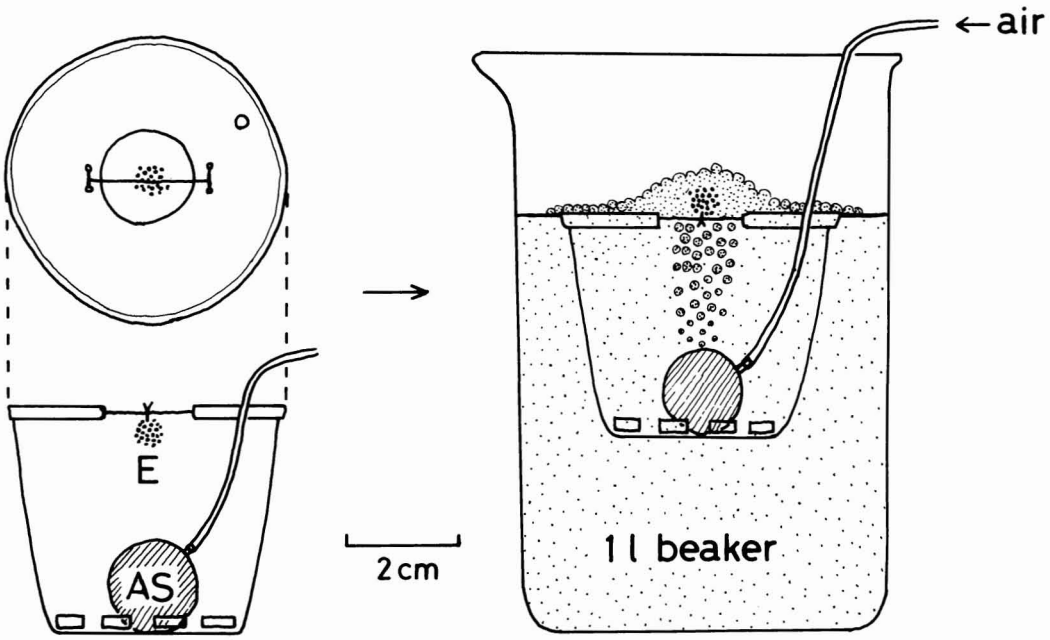


FIGURE 1. A device to provide both aeration and turbulence to eggs detached from the female. E, an egg cluster attached to a portion of the pleopods. AS, air stone. When air is forced into the water, the eggs were floated above the nylon thread, shown on the right.

was splashed with water, she immediately vibrated her body with rapid pumping movements of the abdomen and shook all the larvae into the water. Ovigerous females collected at other times and placed in a similar vessel often exhibited the same kind of behavior, but no hatched larvae appeared at that time. These observations clearly indicate that hatching is not caused by the vigorous pumping movements of the female crab, but is induced as a result of another synchronized process.

Hatching from confined females occurred somewhat later than in free animals: larval release of free animals occurred within 2 hr after sunset, but hatch peaks of the confined females were delayed 1–1.5 hr (Figure 2, lower panel). Time required for hatching in confined females was also longer than in free animals; in some animals it took 10–20 min until the entire sponge became sticky.

Microscope Observations

Eggs attached to a female are at first dark brown because of the accumulation of yolk. Four to 5 days before hatching the amount of yolk rapidly diminishes and the sponge turns brownish green. Egg size at this stage averaged 0.34 × 0.32 mm (in contrast to the estimates of young eggs at 0.2 × 0.28 mm). Well-formed compound eyes, chromatophores, and globules of orange yolk are clearly visible through the transparent egg capsule, as are occasional movements of the body and heartbeat. Swelling, if any, was slight as hatching approached.

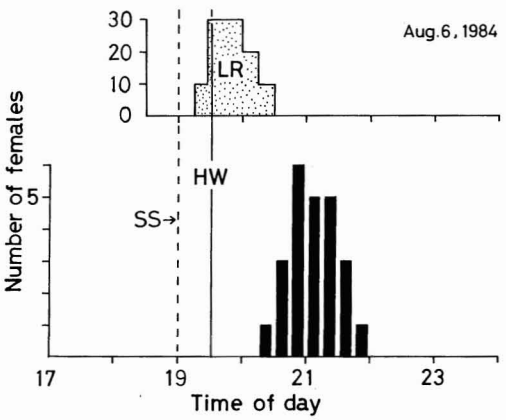


FIGURE 2. Distribution of hatch time recorded in the field at Kasaoka. Collection of females was made at 1830–1900 hours. Vertical straight lines (HW) indicate the time of night high water, SS the time of sunset. The vertical axis of the upper panel shows relative number of females (LR) releasing larvae at night (see Saigusa 1982 for larval release activity by the female; although time of larval release by the population was examined in the field, time of day of release in each female was roughly recorded in 1984).

Before rupture of the egg membrane, the abdomen of the embryo curled ventrally, and there was no fluid-filled space between the body and the egg case. Hatching commenced with the sudden rupture of the egg capsule. No motion of the body was observed at that time. In Figure 3*a*, the egg capsule of larva D has already ruptured, and the larva remains within the broken membrane. Larva C also seems to have sloughed off the egg case, but others have not yet hatched. Hatching of larva B is visible in Figure 3*b*, and that of larva A in Figure 3*c*. There is a slight but obvious difference in relative positions of both eyes (big black spots) in larva A before and after hatching caused by a rotation of the larva associated with breakage of the egg membrane. This motion stopped when the end of the telson was obstructed by the broken membrane. Hatched larvae remain in the folded abdomen of the female until she shakes them off into the water.

When the egg capsule ruptures, the second, thin membrane enclosing the larva is sometimes seen to remain intact (Figure 4*a*). Observations in nonaerated water show that larvae emerging from the egg case remain motionless (e.g., larva D in Figure 3*a–d*) except for strong reverse peristalsis in the rectum, which appeared to be associated with an influx of water. As reverse peristalsis proceeds, the larval body swells and the dorsal spine extends. When the thoracic appendages and telson are fully developed, the larva begins to swim (Figure 4*b*).

Observations by SEM also indicated that the empty egg case consists of two membranes, an outer thick membrane serving as the protective shell of the egg (OM in Figure 5*a* and 5*c*) and an inner, thin membrane lining the egg case (LM in Figure 5*c*). In addition, a

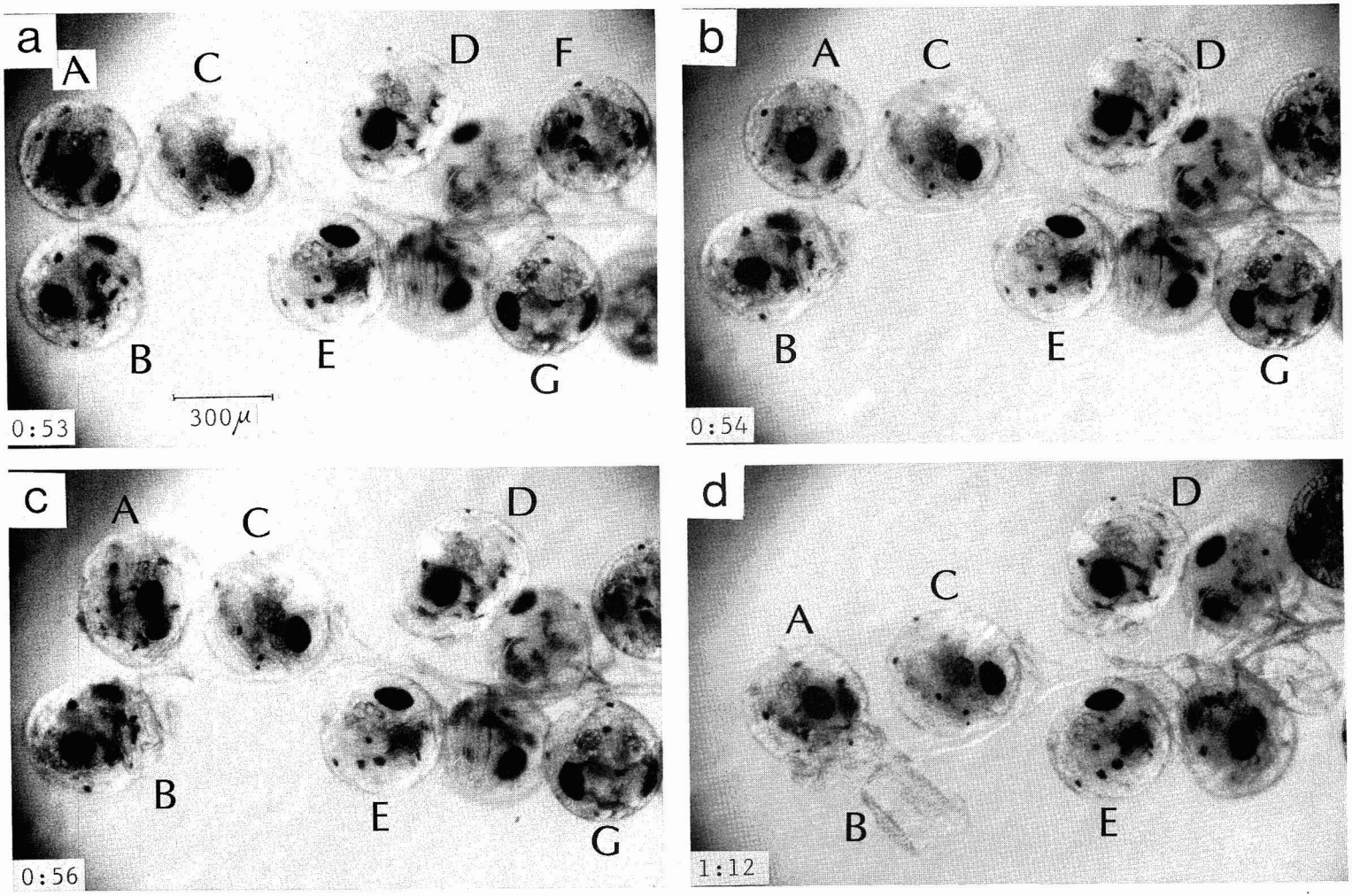


FIGURE 3. Hatching of detached embryos in *S. haematocheir*. Note the progress of embryos A and B. Hatching of the sponge carried by the mother crab occurred at 2315 hours on 26 July 1985. Time in minutes is shown at lower left in each figure (*a–d*). See text for further details. Time of detachment was about 2200 hours on 26 July.

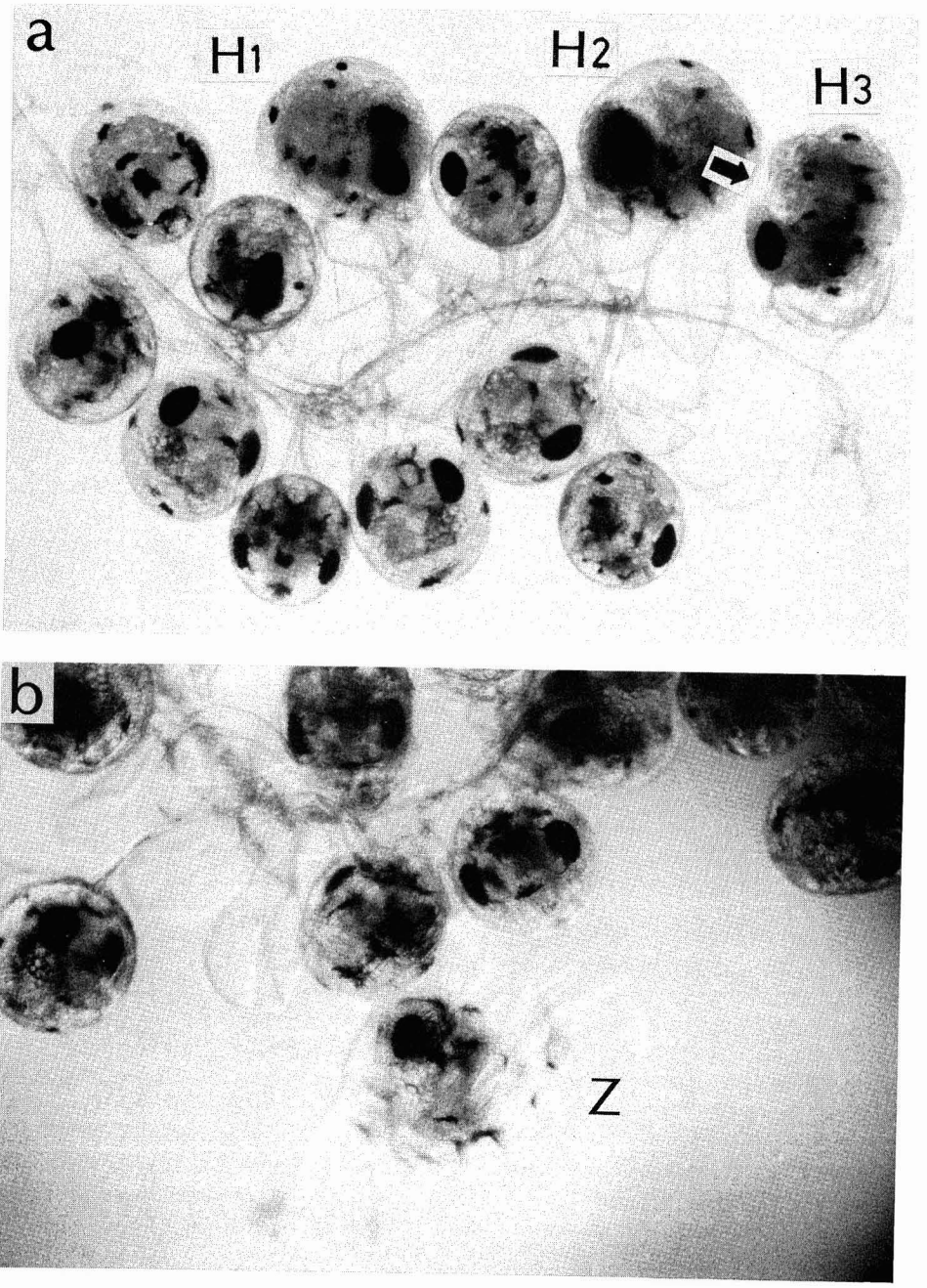


FIGURE 4. Hatching of *S. haematocheir* eggs: (*a*) increase in volume following rupture of the outer membrane (H_1, H_2, and H_3). Arrow shows a delicate, thin membrane enclosing the larva after bursting of the egg case. Other embryos have not yet hatched. (*b*) zoea larva (Z) that has begun to swim after throwing off the thin membranes.

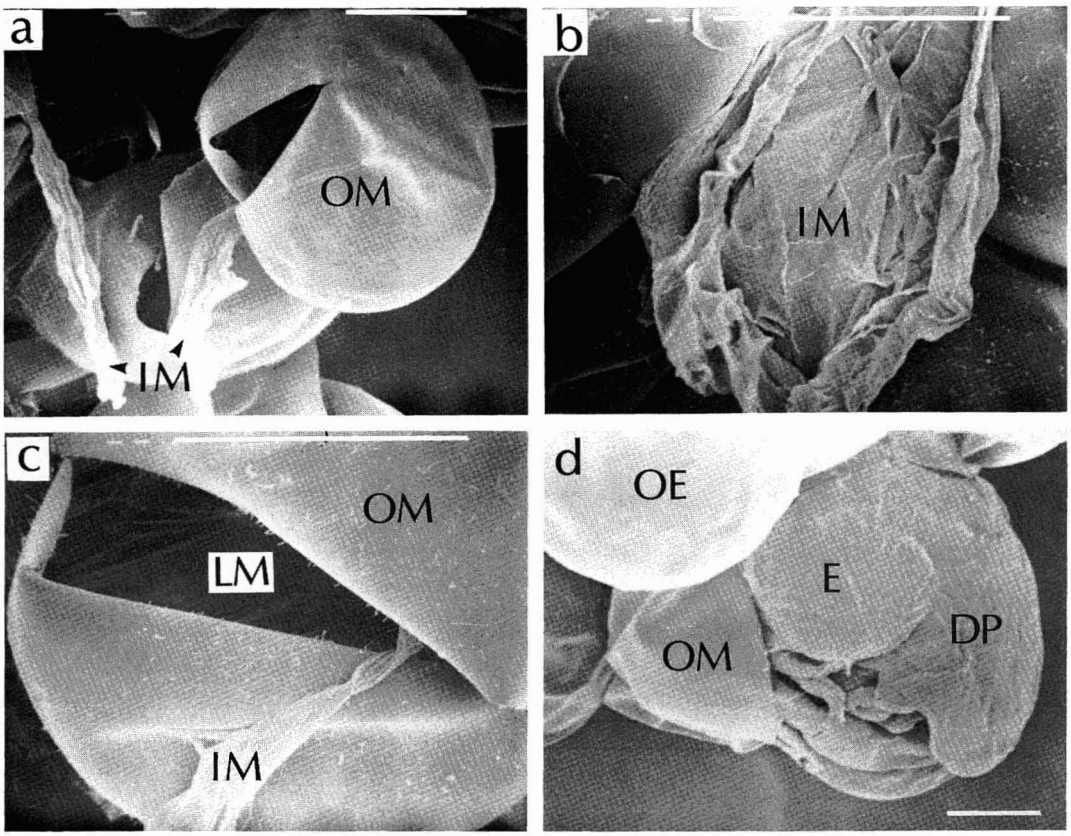


FIGURE 5. Micrographs obtained by SEM: (*a*) egg cases from which hatching occurred and zoeae escaped. OM, outer membrane. IM, the third membrane. (*b*) magnification of the third membrane (IM). (*c*) an empty egg capsule showing the thin lining membrane (LM). (*d*) a prezoea larva after the breakage of the outer membrane. E, a compound eye. DP, dorsal portion of the larva. OE, another egg for which hatching has not yet occurred. Scales expressed in each figure are all 100 μm.

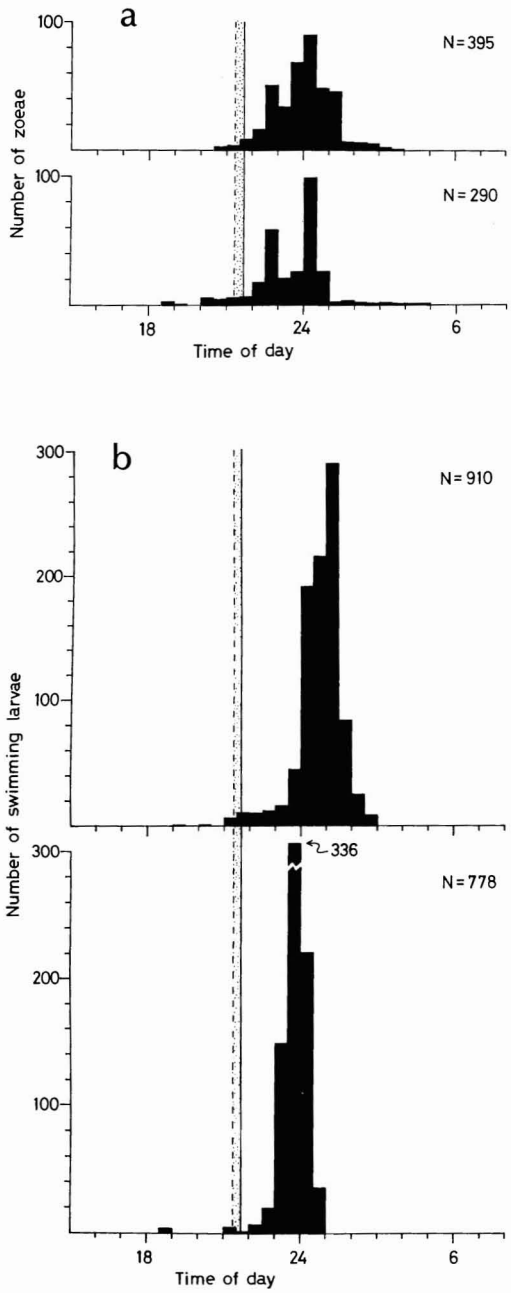
third membrane with an irregular structure (IM) protruded from the empty egg case (Figure 5*a–c*). This is not a so-called embryonic cuticle cast off by the larva, but is a third membrane investing the embryo. This structure can also be recognized with a stereomicroscope, and its presence and sticky nature are suggested by behavior of the larvae after hatching in that the larvae are released only after vigorous movement during which the abdomen is repeatedly straightened.

No evidence was obtained from the SEM views that the egg capsule was dissolved; rather, the outer membrane was clearly cracked on breakage (Figure 5*a* and *c*; Masamichi Yamamoto and Toshiki Makioka, pers. comm.). The larva bulges from the broken egg case (Figure 5*d*) showing that half of the membrane formed a cuplike case over the telson—which also indicates a rupture of the outer membrane. In Figure 5*d*, in which the cephalic portion of the larval body is directly exposed to the outside, the third membrane may already have been shed (or might have been removed during fixation or other experimental manipulation).

Hatching of Detached Embryos

A hatching profile of detached embryos maintained in nonaerated water is shown in Figure 6*a*. Hatching started shortly before

larval release began and continued all night. Hatching peaks occurred 30 min after midnight in two experiments. Hatching synchrony of detached embyros was considerably improved by exposing them to strong aeration and turbulence (Figure 6*b*). For the embryos separated on 5 September (upper panel), most larvae (81%) hatched during the 2-hr interval between midnight and 0200 hours. Embryos detached on the day of larval release (lower panel) showed somewhat better synchrony in hatching: 95% of hatching was concentrated in 2 hr from 2300 to 0100 hours. Furthermore, Figure 6*b* shows a difference in timing between the two experiments: the peak of hatching in the embryos detached on the day of larval release occurred about 1.5 hr earlier than those separated 1 day before. Additional experiments confirmed the initial observations.



Yet another feature of the results shown in Figure 6*a* and 6*b* is a difference in the percentage of the hatching larvae in nonaerated and aerated water. Almost all embryos detached on the day of larval release and maintained in nonaerated water hatched that night. In contrast, only some of the embryos separated 1 day before larval release and maintained in the same conditions hatched (Table 1, uppermost column). The bodies of those embryos that did not hatch were cloudy and apparently motionless. Embryos maintained in aerated water did not show these features (Table 1, lower columns). The implication of these observations is that the eggs may have died because of an oxygen deficiency.

DISCUSSION

Observations by stereomicroscope indicate that the egg cases of *Sesarma* consist of two membranes, a thick outer membrane and an inner thin membrane, as in other decapods (e.g., Yonge 1937). In addition to these mem-

FIGURE 6. Distribution of hatching of eggs separated from a female. (*a*) hatching monitored in still water. (*b*) hatching under strong aeration. Eggs were all detached from one female. Time of separation was 1600 hours on 5 September 1984 (upper panel) and 1530 hours on 6 September 1984 (lower panel) in both illustrations. Time of day of larval release by the female (vertical straight line) was at 2140 hours on 6 September (a portion of hatched larvae began to swim in the vessel 20–30 min before the release of larvae, as shown by the dotted area). N, total number of hatched larvae in each experiment.

TABLE 1

COMPARISON OF HATCHING OF DETACHED EMBRYOS IN DIFFERENT CONDITIONS

EXPERIMENTAL CONDITIONS	DATE OF DETACHMENT	NUMBER OF SWIMMING LARVAE	NUMBER OF EMBRYOS UNHATCHED*	PERCENTAGE (%)
Still water				
	1600 hours on 5 September	395	170	70
	1530 hours on 6 September	290	2	99.3
Aeration and turbulence				
	1600 hours on 5 September	910	2	99.8
	1530 hours on 6 September	778	1	99.9

*A mass of detached embryos included some in which development was extremely delayed. Such embryos did not hatch, and they were excluded from counting.

branes, another thin membrane lining the outer membrane (LM in Figure 5) is clearly visible by SEM. The process of egg-membrane rupture is also similar in several respects to that described for other decapod crustaceans (Burkenroad 1947, Davis 1964, 1965, 1968). From SEM studies (Figure 5), egg hatching in *Sesarma* appears to result from mechanical rupture of the egg capsule. The pressure causing rupture comes from within the egg and not from the female. The pressure may be exerted by the swelling embryo itself, perhaps associated either with the uptake of water (Davis 1964), or it may result from osmotic swelling of the inner membrane enclosing the embryo (Davis 1965).

In *Sesarma* the evidence for rupture of the egg membranes by the embryo itself lies in the following observation: in the eggs that have hatched, the larval body swells (H_1, H_2, and H_3 in Figure 4*a*), and a strong reverse peristalsis is observed. Increased pressure within the egg capsule, therefore, may result from water reaching the embryo as the female circulates water over the egg sponge with her pleopods when she moves about on land. The difficulty with this hypothesis is that intestinal peristalsis is barely visible under the stereomicroscope in embryos that are squeezed within the rigid outer membrane before hatching.

Another possible explanation for membrane breakage is that a sudden increase in excretion by the embryo leads to increased salt content and consequent influx of water surrounding the embryos. In many species an increase in volume takes place inside the inner membrane (Marshall and Orr 1954, and a review by Davis 1968). In *Sesarma*, however, it is difficult to see how osmotic processes would be involved: larval release occurs in water of various salinities from seawater to fresh water (Saigusa 1982, 1985), and the females go in and out of water while they carry egg sponges. If the osmotic explanation is correct, it would be necessary both to show the bulging of the thin membrane and to explain the absence of a fluid-filled space between the inner membrane and the swelling embryo even after breakage of the outer membrane.

In short, egg hatching in *Sesarma* is not fully accounted for by either hypothesis. Hatching in this crab obviously includes temperature-dependent factors, and there are still many other (unpublished) phenomena that have to be explained.

The problem of synchronization of hatching in the large number of embryos carried by the female *S. haematocheir* has not been solved either. Forward and Lohmann (1983) reported that improved hatching timing in the estuarine crab *Rhithropanopeus harrisii* occurs in turbulent water when the females rapidly and vigorously pump their abdomens. Groups of

larvae are released with each pump, and those authors suggested that pumping behavior itself assists in breaking the egg membrane, which enhances hatching synchrony. In *Sesarma*, however, no particular movements of the female's abdomen are observed upon egg hatching.

What is evident is that hatching synchrony is enhanced by aeration and water turbulence (Figure 6), there is less hatching synchrony in detached eggs than in the eggs attached to the female (although hatching synchrony improves somewhat when eggs are detached on the day of release), and a hatch peak of confined crabs is delayed (Figures 2 and 6). As indicated from Figures 3 and 4, some of the larvae with outer membrane breakage had difficulty in leaving the egg capsule. So detached embryos exposed to vigorously aerated water may have been readily released from the egg capsules, which would cause a better synchrony of hatching. Figure 6 also indicates that hatching synchrony of the detached eggs was decreased and their hatch time delayed. These features can be explained in terms of the absence of some cue from the female. And the delay of hatching in confined crabs (Figure 2) may imply a delay of this cue.

Since egg hatching time is surprisingly short for the eggs attached to the female, it might be possible that a substance similar to hatching enzyme secreted by either the embryo or the female, and which softens the egg membrane, resulting in simultaneous hatching, is present. In addition, it appears that in *Sesarma* embryos a hatching process different from embryonic development is involved, and both mechanisms for the commencement of this process and for synchronization of hatching must be considered in accounting for the breakage of the egg case.

The adaptive significance of the hatching system is evident for estuarine terrestrial crabs such as *Sesarma*. Because larval release occurs at the water's edge, synchronization of hatch time ensures that all the larvae are released at the same time. Hatching just before liberation also ensures that the larvae will not suffer oxygen deficiency and other adverse effects that might occur if they are held by the female for a longer time.

ACKNOWLEDGMENTS

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